Ocular vestibular evoked myogenic potentials as a test for myasthenia gravis

Valko, Yulla ; Rosengren, Sally M ; Jung, Hans H ; Straumann, Dominik ; Landau, Klara ; Weber, Konrad P

Abstract: OBJECTIVE To explore whether ocular vestibular evoked myogenic potentials (oVEMP) can be used to detect a decrement in the extraocular muscle activity of patients with myasthenia gravis (MG). METHODS Twenty-seven patients with MG, including 13 with isolated ocular and 14 with generalized MG, and 28 healthy controls participated. We applied repetitive vibration stimuli to the forehead and recorded the activity of the inferior oblique muscle with 2 surface electrodes placed beneath the eyes. To identify the oVEMP parameters with the highest sensitivity and specificity, we evaluated the decrement over 10 stimulus repetitions at 3 different repetition rates (3 Hz, 10 Hz, and 20 Hz). RESULTS Repetitive stimulation at 20 Hz yielded the best differentiation between patients with MG and controls with a sensitivity of 89% and a specificity of 64% when using a unilateral decrement of 15.2% as cutoff. When using a bilateral decrement of 20.4% instead, oVEMP allowed differentiation of MG from healthy controls with 100% specificity, but slightly reduced sensitivity of 63%. For both cutoffs, sensitivity was similar in isolated ocular and generalized MG. CONCLUSION Our study demonstrates that the presence of an oVEMP decrement is a sensitive and specific marker for MG. This test allows direct and noninvasive examination of extraocular muscle activity, with similarly good diagnostic accuracy in ocular and generalized MG. Thus, oVEMP represents a promising diagnostic tool for MG. CLASSIFICATION OF EVIDENCE This study provides Class III evidence that oVEMP testing accurately identifies patients with MG with ocular symptoms (sensitivity 89%, specificity 64%).

DOI: https://doi.org/10.1212/WNL.0000000000002383

Ocular vestibular evoked myogenic potentials (oVEMP) as a test for myasthenia gravis

Yulia Valko, MD[1,2], Sally M. Rosengren, PhD[3,4], Hans H. Jung, MD[2], Dominik Straumann, MD[2], Klara Landau, MD FEBO[1], Konrad P. Weber, MD[1,2]

[1]*Department of Ophthalmology, University Hospital Zurich, University of Zurich, Switzerland*
[2]*Department of Neurology, University Hospital Zurich, University of Zurich, Switzerland*
[3]*Department of Neurology, Royal Prince Alfred Hospital, Sydney, Australia*
[4]*Central Clinical School, University of Sydney, Sydney, Australia*

Correspondence to:
Konrad P. Weber, MD (conducted the statistical analysis)
Department of Ophthalmology, University Hospital Zurich, University of Zurich
Frauenklinikstrasse 24, 8091 Zurich, Switzerland
Tel. +41 44 255 11 11; Fax +41 44 255 43 49; Email: konrad.weber@usz.ch

Email addresses: yulia.valko@usz.ch; sally@srosengren.org; hans.jung@usz.ch; dominik.straumann@usz.ch; klara.landau@usz.ch

Character count title:	84
Abstract word count:	243
Text word count:	3000
Tables:	1
Figures:	4
References:	34

Supplemental Table, electronic file name: table e-1
Supplemental Figure, electronic file name: figure e-1
Supplemental Video: video 1

Search terms: [179] Myasthenia, [194] Diplopia (double vision), [285] EMG, [193] Eyelids, [187] Ocular motility

Author contributions:

Yulia Valko collected and analyzed the data and wrote the manuscript.

Sally Rosengren contributed to the study design, data analysis and writing of the manuscript.

Hans Jung contributed to the patient examination and review of the manuscript.

Dominik Straumann contributed to the study design, data analysis and review of the manuscript.

Klara Landau contributed to the study design, patient examination and review of the manuscript.

Konrad Weber designed the study, contributed to data collection, data analysis and writing of the manuscript, and conducted the statistical analysis.

Study funding: the study was supported by a research grant of the University of Zurich (Forschungskredit der Universität Zürich).

Disclosure Agreement: All authors report no disclosures.

Abstract

Objective: To explore whether ocular vestibular evoked myogenic potentials (oVEMP) can be used to detect a decrement in the extraocular muscle activity of myasthenia gravis (MG) patients.

Methods: Twenty-seven MG patients, including 13 with isolated ocular and 14 with generalized MG and 28 healthy subjects participated. We applied repetitive vibration stimuli to the forehead and recorded the activity of the inferior oblique muscle with two surface electrodes placed beneath the eyes. To identify the oVEMP parameters with the highest sensitivity and specificity, we evaluated the decrement over 10 stimulus repetitions at three different repetition rates (3Hz, 10Hz and 20Hz).

Results: Repetitive stimulation at 20Hz yielded the best differentiation between MG patients and controls with a sensitivity of 89% and a specificity of 64% when using a *unilateral* decrement of ≥15.2% as cut-off. When using a *bilateral* decrement of ≥20.4% instead, oVEMP allowed differentiation of myasthenia gravis from healthy controls with 100% specificity, but slightly reduced sensitivity of 63%. For both cut-offs, sensitivity was similar in isolated ocular and generalized MG.

Conclusions: Our study demonstrates that the presence of an oVEMP decrement is a sensitive and specific marker for MG. This test allows direct and non-invasive examination of extraocular muscle activity, with similarly good diagnostic accuracy in ocular and generalized MG. Thus, oVEMP represents a promising diagnostic tool for MG.

Classification of Evidence: This study provides Class III evidence that oVEMP testing accurately identifies patients with MG with ocular symptoms (sensitivity 89%, specificity 64%).

Abbreviations: AChR = acetylcholine receptor; MG = myasthenia gravis; RNS = repetitive nerve stimulation; oVEMP = ocular vestibular evoked myogenic potentials; IO = inferior oblique muscle.

Introduction

Myasthenia gravis (MG) is a chronic autoimmune disorder characterized by defective neuromuscular transmission.[1] The clinical hallmark is a fluctuating, exercise-dependent and usually reversible muscle fatigability. About 85% of the patients initially demonstrate only ocular symptoms, but the disease generalizes in up to 80%, usually within 1 year.[2] Early diagnosis of this potentially lethal disorder is important,[3] as early immunosuppressive treatment of ocular MG may reduce the risk of generalization.[4,5] Nevertheless, up to 46% of patients do not receive the correct diagnosis within the first year of onset.[6,7]

The most common ancillary tests include antibody assays, electrophysiological tests (repetitive nerve stimulation, RNS or single-fiber electromyography, SFEMG) and edrophonium test. In isolated ocular MG, however, these tests exhibit a reduced sensitivity.[8]

Correct evaluation of ocular symptoms is critical for shortening the diagnostic delay in MG. However, the extraocular muscles have previously not been directly accessible for clinical testing. We therefore assessed an additional application of a vestibular test – ocular vestibular-evoked myogenic potentials (oVEMP) – for measuring the pathognomonic decrement of extraocular muscle activity in MG. OVEMPs are a recently developed test that records electromyographic activity of the inferior oblique muscle

(IO).[9-13] Originally, the test was designed to assess otolith function.[14,15] Here, we set out to demonstrate the utility of oVEMP to detect the decrement in extraocular muscle surface-EMG of MG patients. Compared to other tests, oVEMP would be a unique tool in ocular MG, as it may *directly* and *non-invasively* reveal the pathognomonic fatigability of extraocular muscles.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This prospective study was conducted at the Departments of Ophthalmology and Neurology, University Hospital Zurich, Switzerland. The Canton Zurich Ethics Committee approved the protocol (KEK-ZH-Nr 2010-0177). We obtained informed written consent from all participants and an authorization-for-disclosure for figure and video from all recognizable participants in accordance with the Helsinki Declaration. The primary purpose of the study was to provide Class III evidence that oVEMP accurately differentiates between MG patients with ocular symptoms and healthy subjects.

Subjects

We included 27 MG patients (mean age 58, range 26-86) with ocular involvement **(table e-1)**. All patients had ocular symptoms, including ptosis (100%) and diplopia (85%). Thirteen patients (48%) had isolated ocular MG and 14 patients (52%) generalized MG. Diagnosis of MG was made based on the presence of a typical history and at least one positive ancillary test, including edrophonium testing, RNS and serum autoantibodies (anti-AChR, anti-Titin, anti-MuSK). We included a control group of 28 healthy subjects (mean age 47, range 21-86) without any neuromuscular, vestibular or oculomotor

disorders. All patients but one were already on pyridostigmine treatment, which was interrupted overnight in 21 patients and 4-5 hours prior to testing in 5 patients. We did not include subjects with known vestibulocochlear disorders, as oVEMP signals may not be elicited in these patients. The oVEMP results of three control subjects and one patient were not analyzable due to excessive blink artifacts and were excluded.

Equipment

Subjects lay supine with their head on a pillow. The skin was cleaned with abrasive gel (Nuprep, USA) before surface electrodes (Blue sensor N, Ambu, Denmark) were applied beneath the eyes. For each eye an active electrode was placed over the infra-orbital margin and a reference directly below on the cheek **(figure 1)**. We placed a ground electrode on the chin. OVEMPs were elicited by bursts of bone-conducted skull vibration delivered with a hand-held 'minishaker' positioned over the hairline (minishaker 4810; amplifier 2706, Bruel and Kjaer, Naerum, Denmark) **(video 1)**. Stimulus generation and surface EMG recording was done with laboratory data acquisition devices (power 1401, 1902 pre-amplifier, CED, UK). Signals were bandpass filtered (5Hz-2000Hz) and sampled at 10kHz with sweep-based recording software (Signal, version 5, CED, UK). To reduce possible electro-magnetic interference with the recordings, the minishaker was shielded with a µ-Metal encasement **(figure 1)**.

Recording

We delivered 4ms bursts of 500Hz bone-conducted vibration in trains of ten stimuli **(video 1)**. The ten stimuli were given at three different repetition rates of 3, 10 and 20Hz. We usually started with the 3Hz paradigm and ended with the 20Hz paradigm. To avoid fatigue during the test, we ensured that the patients had breaks of at least 2

minutes between tests. For each paradigm we delivered 20-30 repetitions of trains with ten stimuli while the subjects were looking up. Trains were separated by 5s of rest, during which subjects closed their eyes for 3s.

The oVEMP signal consists of at least two peak-trough sequences **(figure 2)**. The first peak-trough (n10-p15) is a vestibular-dependent reflex of the IO originating from the contralateral ear.[9] The second peak-trough is also predominantly vestibular, but probably originates from both ears.

Data processing and statistics

The study results are reported in accordance with the Standards for Reporting of Diagnostic Accuracy Studies (STARD) **(figure e-1).** Analysis of oVEMP data was performed using MatLab (MathWorks, Natick, MA, USA). Residual main artifacts were removed using a 50Hz notch filter (Q factor=10), occasional electrooculographic eye movement artifacts after the first trial were removed using a 4^{th}-order Butterworth high-pass filter with a 20Hz cutoff-frequency. Minimal distortion of the filtered oVEMP signal was ensured by comparison to the raw signal. Signal repetitions were averaged after outliers were removed with a median absolute deviation (MAD) algorithm, complemented by visual inspection. Mean rejection rates in patients and controls were similar (18±14% vs. 15±11%).

Statistical analyses were performed using IBM SPSS (version 22.0, USA). Group data were described by means and standard deviations, unless otherwise specified. For average comparison of normally distributed data, we used unpaired Student's *t*-tests. To compare mean decrements between more affected and less affected eyes, we used paired *t*-tests.[16] The chi-square test was used for nominal data. For non-parametric data, we applied the Mann-Whitney *U*-test. We calculated Spearman's rank coefficient

for correlation analysis of non-normally distributed data. To determine optimal oVEMP cut-off values, we applied receiver operating characteristics (ROC). Significance was accepted at $p<0.05$.

Results

Patient characteristics

Table e-1 gives an overview on demographic, clinical and diagnostic characteristics of the 27 MG patients. AchR-antibodies were positive in 20/27 tested patients (74%), titin-antibodies in 8/23 tested patients (35%), and MuSK-antibodies in 1/21 tested patients (5%). Seven patients (26%) had negative results in all three antibody assays, with six of them having isolated ocular MG. Edrophonium test was positive in 16/18 patients (89%), while one patient had only subjective improvement of diplopia and one showed no improvement of diplopia. Both of these patients (11%) had isolated ocular MG. In 7 patients (26%) CT thorax revealed thymoma. All of them, and 4 patients without thymoma, underwent thymectomy (11 patients, 41%).

OVEMP in MG

OVEMP responses were present in all patients and controls. They consisted of a series of peaks and troughs and the second peak and trough were generally largest. Therefore we used the peak-to-peak amplitude of this second biphasic wave to measure any decrement over the stimulus trains. **Figure 2** illustrates oVEMP recordings from an MG patient and a control subject explaining the decrement calculation method. We defined the decrement as the difference between the second oVEMP response and the average of the fifth to ninth responses. The first oVEMP response showed an increased

amplitude with high variability in healthy controls. Therefore we used the second response as reference. Next we explored which repetition rate was best to discern patients from controls **(figure 3)**. Mean decrements did not differ between patients and controls when stimulating with 3Hz (-11.6%±17.8 vs. -7.7%±15.4, p=0.23) and 10Hz (-5.4%±28.1 vs. -1.7%±16.7, p=0.41). Conversely, at 20Hz the mean decrement in patients was larger than in controls (-21.5%±29.6 vs. -2.8%±16.9, p<0.001). As shown in **table e-1**, interindividual variability in decremental responses of patients was high, but intraindividual correlation (left vs. right eye) of oVEMP decrements was robust (r=0.614, p=0.001).

Using ROC analysis, we identified optimal decrement cut-offs for distinguishing patients from normal subjects **(figure 4)**. To account for the fact that each subject contributed two measurements (one for each eye)[16], we explored two different decrement types: *unilateral* decrement (where at least one of the two eyes showed a decrement) and *bilateral* decrement (where both eyes showed a decrement). For the *unilateral* analysis, only the eye with the *larger* decrement was considered. For the *bilateral* analysis, only the eye with the smaller decrement was considered, as this eye sets the threshold for bilateral involvement. Stimulation at 20Hz had superior diagnostic yields than 3Hz and 10Hz. ROC analysis revealed that in 20Hz trains a *unilateral* decrement of ≥15.2% had a sensitivity of 89% and a specificity of 64% (24 patients and 10 controls with unilateral decrement ≥15.2%, p<0.001). Instead, when using a *bilateral* decrement of ≥20.4% as cut-off, specificity was 100% and sensitivity 63% (17 patients with bilateral decrement ≥20.4% and 0 controls, p<0.001). There was no correlation between the magnitude of decrement and age, and mean age was similar in patients with and without bilateral decrement (59±17 vs. 55±17, p=0.49).

Isolated ocular MG

The diagnostic yield of antibody assays and RNS was lower in patients with isolated ocular MG compared to patients with generalized MG (**table 1**). The frequency of positive oVEMP findings, however, was similar in both groups. Specifically, twelve patients with isolated ocular MG and twelve patients with generalized MG showed a *unilateral* decrement of ≥15.2%, yielding a comparable sensitivity of 92% and 86% (p=1.00). Likewise, a *bilateral* decrement of ≥20.4% showed a sensitivity of 62% in ocular and 64% in generalized MG (p=0.60).

Clinical symptoms and oVEMP findings

In all but one patient, ptosis was asymmetric, with unilateral predominance in 16 patients and strictly unilateral manifestation in 11 patients. MG patients had more pronounced oVEMP decrements on the clinically (more) affected eye compared to the less or not affected eye (-27.4%±26.0 vs. -16.2%±33.2, p=0.03). On the other hand, patients without overt ophthalmoparesis (n=4) had similar oVEMP findings as those with diplopia (n=23) (-18.8±32.2% vs. -21.9±29.5%, p=0.79). In two patients the clinical involvement was evident in one eye only (unilateral ptosis, no diplopia), but oVEMP revealed a bilateral decrement ≥20.4% in both cases.

Discussion

This is a proof of principle study with Class III evidence that oVEMP testing accurately identifies MG patients with ocular symptoms. We have demonstrated that a reflex of the extraocular muscles decreases with repetitive vestibular stimulation using oVEMP technique, similar to RNS. The test yielded a sensitivity of 89% and a specificity of 64% with a *unilateral* decrement of ≥15.2%. Instead, when using a *bilateral* decrement of ≥20.4% as cut-off, oVEMP allowed differentiation between MG and healthy controls with 100% specificity, however at the cost of reduced sensitivity of 63%. While traditional diagnostic procedures suffer from reduced sensitivity in ocular compared to generalized MG,[8,17] the sensitivity of oVEMPs appears equally high in isolated ocular MG, as this test *directly* assesses the decrement in extraocular muscle EMG. Specifically, 12/13 patients with isolated ocular MG and 12/14 patients with generalized MG showed a *unilateral* decrement of ≥15.2%, yielding a comparable sensitivity of 92% and 86%. Similarly, a *bilateral* decrement of ≥20.4% showed a sensitivity of 62% in ocular and 64% in generalized MG. Hereby, we demonstrated the diagnostic utility of oVEMP in MG with ocular involvement.

The oVEMP has only recently been introduced into clinical practice to test otolith function in vestibular disorders. The test is based on the vestibulo-ocular reflex (VOR) and allows examination of brisk extraocular muscle activation after vestibular otolith stimulation.[11,13,14] Most probably, all extraocular muscles receive neural projections from the otoliths. However, using simultaneous surface and needle recordings of the IO, we recently identified the IO as the principal origin of the excitatory potentials measured by the surface recordings in the clinical oVEMP.[9] Any structural lesion between otoliths and extraocular muscles likely affects oVEMP characteristics. Clinical application has

produced two types of pathological oVEMP results: 1) the amplitude of potentials may be reduced or absent as in acute vestibular neuritis[18], or 2) the dynamics of potentials may be delayed as in inflammatory or neurodegenerative CNS conditions (multiple sclerosis or Parkinson's disease).[19,20] The present study, however, demonstrates a third electrophysiological dimension of oVEMPs, namely the capability of measuring the pathognomonic decrement in extraocular muscles of MG patients.

Using ROC analysis we found that repetitive stimulation with 20Hz trains provided higher discriminatory power than lower repetition rates (3Hz and 10Hz trains). The possibility to apply fast repetition rates is one important advantage of oVEMP, which is not possible by measuring voluntary saccadic eye movements.[21,22] As a consequence, oVEMP allowed us to unmask myasthenic decrements even in clinically asymptomatic eyes. We defined the oVEMP decrement as the reduction between the second stimulus and the average of the fifth to ninth stimuli. For reasons to be investigated, the first stimulation produced an increased amplitude with high variability in healthy controls, while the second response was sometimes larger than the first at high repetition rate in MG patients. Superimposition of subtle blink artifacts may in part account for this effect, although analysis of all patients' and controls' curves demonstrated such effects also in the absence of obvious blink artifacts. The fluctuations observed during the first two stimuli may reflect facilitation effects, possibly caused by acetylcholine quantal release.[23] For these reasons, we preferred to use the second peak as reference value to avoid any bias by differently enlarged first peaks.

A critical question concerns the differential discriminatory power of a unilateral vs. bilateral decrement. The advantage of unilateral decrement is gain in sensitivity, which may be particularly desirable when evaluating patients with very mild symptoms. On the other hand, the advantage of using a bilateral decrement is its high specificity, which is

on a par with the 94-99% specificity described for other methods including antibody assays and RNS.[24] The cause of occasional unilateral decrements in controls remains unclear, but artifacts due to positioning of the mini-shaker should be considered. Little is known about the variability in disease severity between individual extraocular muscles, but our oVEMP measurements may have failed to detect a decrement if the myasthenic process spared the IO. Thus, technical improvements enabling recordings from additional extraocular muscles may improve oVEMP sensitivity in ocular MG even further.

Compared to other diagnostic tests for MG, oVEMP has several merits. Antibody assays and RNS are highly specific, but have low sensitivities in ocular MG. For instance, an overview of six studies assessing the diagnostic accuracy of anti-AChR antibodies found sensitivities of 87-98% in generalized MG but only 39-71% in ocular MG.[24] Similarly, six of our ocular MG patients were “triple-seronegative”, including three patients with no decrement in RNS, yet five of them had positive oVEMP results, thus highlighting the diagnostic usefulness of oVEMP in this challenging subgroup.

The sensitivity of RNS in ocular MG is even lower, ranging between 11-39%.[24] In ocular MG, SFEMG is considered the diagnostic gold standard, as reflected by four studies reporting a high sensitivity of 92-97%[25-28], although two other groups found lower sensitivities of 83% and 62%.[29,30] Comparison of different electromyographic tests in ocular MG demonstrated that the closer the measurement to the extraocular muscles, the higher the sensitivity. In contrast to SFEMG, oVEMP not only directly assess the extraocular muscles, but is also better tolerated, because it is less invasive, less cumbersome and less operator-dependent.

OVEMP also has several advantages over the edrophonium test. Edrophonium testing is limited to patients with observable deficits (e.g. ptosis, diplopia) and is contraindicated

in cardiac or respiratory disease. In addition, the drug is not registered in many countries and therefore often unavailable outside university hospitals.

Our study suggests that oVEMP bypasses many of the above-mentioned limitations, as its diagnostic sensitivity seems less susceptible to the immunological and clinical heterogeneity in MG. In other words, oVEMP gives direct evidence of the myasthenic decrement in affected extraocular muscles, irrespective of the exact underlying immunologic process. Therefore, oVEMP is suitable to become an integral diagnostic part in the routine evaluation of patients with suspected MG.

Since the main purpose of the study was proof of principle, we used a case-control and not a cohort study design. In other words, we provide Class III evidence of the capability of oVEMP to distinguish between MG patients and healthy controls, but future studies will need to confirm its diagnostic utility in clinical practice, when the main challenge is differentiation from patients with other neuro-ophthalmological conditions. Second, we studied patients with previously established diagnoses because we wanted to validate our oVEMP measurements in MG. A consequence was that all patients but one were already under treatment with cholinesterase inhibitors, although oVEMP was usually performed in the morning prior to the first drug intake of the day. Therefore it remains unclear what the diagnostic accuracy of oVEMP would be in drug-naïve patients, though it can be speculated that more pronounced muscle fatigability would actually enhance the sensitivity of oVEMP, similar to the treatment effects on RNS in generalized MG.[31,32] Third, ophthalmoparesis was rather mild in our patients and it remains to be determined to what extent severe limitation in upward gaze may interfere with oVEMP testing. Fourth, we did not randomize the sequence of the tests, but performed the 3Hz test first, and the 20Hz test at the end. Nevertheless, the tests were short and patients had breaks between the tests, so that a potential sequence-induced bias due to a

cumulative fatigue effect should be minimal. Finally, our control group was slightly younger, and small age-related effects on oVEMP have been described.[33,34] Nevertheless, we did not find any correlation between the magnitude of decrement and age, and mean age was similar in patients with and without bilateral decrement.

Our findings indicate that oVEMP has the potential to play a helpful role in the diagnosis of ocular MG. Compared to other diagnostic procedures, oVEMP has the unique advantage of providing direct evidence of the myasthenic process in affected extraocular muscles. Technical advances and optimization in signal detection from as many different extraocular muscles as possible will likely further increase the sensitivity of oVEMP in MG. Finally, oVEMP is a simple, non-invasive and fairly inexpensive tool that can be linked to any standard EMG equipment and therefore easily implemented in any clinical electrophysiology unit.

Acknowledgment

The study was supported by a research grant of the University of Zurich (Forschungskredit der Universität Zürich). The authors thank the Betty and David Koetser Foundation for Brain Research, the Albert Bruppacher Foundation for Eye Research and the OPOS Foundation for support. We are grateful to Marco Penner for technical support (Department of Neurology, University Hospital Zurich, Switzerland).

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Figure legends

Figure 1. Experimental setup for oVEMP recording.

Recording is performed in sustained upgaze as in the 'Simpson test' thus activating the superior rectus and inferior oblique muscles in both eyes. A minishaker at the hairline of the forehead delivers the bone-conducted vibration stimulus. Active (*black*) and reference (*red*) electrodes measure the surface EMG signal from the inferior oblique muscles; ground electrode (*green)* is on the chin.

Figure 2. OVEMP recordings in a myasthenic patient (A) and in a normal subject (B).

The grey bars indicate the 10 stimuli at 20Hz repetition rate, which are followed by characteristic oVEMP signals. OVEMP signals are averaged from 20-30 responses. The magnitude of each oVEMP signal was calculated as the sum of the second peak and second trough (asterisks). The magnitude of the decrement (C) was calculated as the difference between the amplitude of the second stimulation and the mean amplitude of the fifth to ninth stimulations.

Figure 3. Group comparison between MG patients and controls at three different repetition rates.

The mean oVEMP responses of the first nine stimulations are shown for each paradigm. Each oVEMP response is indicated as normalized value ± 1 SD with respect to the second stimulation as reference. Mean decrement was similar in MG patients *(red)* and controls *(blue)* when applying trains of 3Hz (A) and 10Hz (B). Only repetitive stimulation at 20Hz (C) allowed reliable group differentiation, as indicated by the significantly larger decrement in MG patients than in controls.

Figure 4. Receiver-operating characteristic curve (ROC) analyses to determine optimal cut-off.

The box plots in the upper row compare the distribution of subjects. The dotted red lines indicate the optimal diagnostic thresholds for unilateral (A) and bilateral (B) decrement, as derived from the ROC analyses shown in the lower row (C, D). For the unilateral decrement, we analyzed and plotted the eye with the larger decrement (A, C). For the bilateral decrement, we analyzed and plotted the eye with the smaller decrement (B, D), as this eye sets the threshold for bilateral involvement. The boxes show median (red line), first and third quartiles, while the end of the whiskers represent the most extreme data points without outliers. For both unilateral (C) and bilateral (D) decrements, the area under the curve (AUC) was larger at 20Hz (*red*) than at 3Hz (*green*) and 10Hz (*blue*). At 20Hz a unilateral decrement of ≥15.2% has the advantage of a high sensitivity of 89% with only three false-negative cases, while a bilateral decrement of ≥20.4% has an excellent specificity of 100% without any false-positive cases.

Table 1. Comparison of demographical, clinical and diagnostic characteristics between patients with isolated ocular MG and generalized MG. While the diagnostic yield of antibody assays and repetitive nerve stimulation is lower in patients with isolated ocular MG than in generalized MG, the sensitivity of oVEMP does not differ, making it hence a valuable test in patients lacking non-ocular symptoms.

	Isolated ocular MG (n = 13)	Generalized MG (n = 14)	*p*
Age, y	49 ± 14	66 ± 15	**0.01**
Gender, female	6 / 13 (46%)	3 / 14 (21%)	0.17
Disease duration, months	40 ± 63	38 ± 43	0.93
Diagnostic delay, months	0.9 ± 1.2	1.7 ± 2.1	0.24
Antibody assays			
AChR-ab, pos	7 / 13 (54%)	13 / 14 (93%)	**0.03**
Titin-ab, pos	1 / 10 (10%)	7 / 13 (54%)	**0.04**
MuSK-ab, pos	0 / 9	1 / 12 (8%)	0.57
All antibodies neg	6 /13 (46%)	1 / 14 (7%)	**0.03**
Repetitive nerve stimulation			
Positive decrement	3 / 7 (43%)	6 / 6 (100%)	**0.049**
Edrophonium test			
Positive	7 / 9 (78%)	9 / 9 (100%)	0.24
oVEMP findings			
Uni- or bilateral decrement ≥15.2%	12 / 13 (92%)	12 / 14 (86%)	1.00
Bilateral decrement ≥20.4%	8 / 13 (62%)	9 / 14 (64%)	0.60
Decrement, magnitude	-27.2 ± 23.9	-16.1 ± 33.5	0.17

Table e-1 Demographic, clinical and diagnostic characteristics of the 27 patients with myasthenia gravis

Patient	Age / gender	Disease duration	Clinical manifestation ptosis	diplopia	bulbar	limbs	Antibodies AChR	Titin	MuSK	Repet. nerve stim. decr.	nerv	Tensilon test	Thy-moma	Thym-ectomy	Drug treatment	oVEMP-decr. (%) right (20Hz)	left
1*	70 / F	5m	L > R	+	-	-	+	+	n.d.	+	XI	n.d.	-	-	P	**-17.0**	-11.9
2*	49 / F	10m	R / -	-	-	-	+	-	-	+	Uln.	n.d.	-	+	P S	**-21.1**	**-40.4**
3*	66 / F	9m	L > R	+	-	-	+	n.d.	n.d.	-	VII, XI, Uln.	-	-	-	P S A	**-21.0**	**-49.8**
4*	60 / M	4m	- / L	+	-	-	+	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	P	-6.6	**-16.0**
5*	51 / F	4y, 7m	R / -	+	-	-	+	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	P S A	**-30.8**	**-24.2**
6*	28 / M	2y	R / -	+	-	-	+	-	-	n.d.	n.d.	+	-	-	P	**-23.9**	39.1
7*	36 / F	1y, 6m	L > R	+	-	-	+	-	-	n.d.	n.d.	+	+	+	P S	-9.5	10.0
8*	34 / M	4y, 2m	R / -	+	-	-	-	-	-	+	XI	-/+	-	+	P S	**-45.9**	**-34.4**
9*	55 / M	5y, 4m	- / L	+	-	-	-	-	-	-	XI	+	-	-	P S A	12.7	**-63.8**
10*	26 / M	1y	- / L	+	-	-	-	-	-	-	VII	+	-	-	P S A	**-48.2**	**-31.6**
11*	63 / M	20y	R > L	+	-	-	-	-	-	-	VII, Uln.	+	-	+	P S A	**-56.0**	**-45.2**
12*	47 / M	2y, 4m	R > L	+	-	-	-	-	-	n.d.	n.d.	+	-	-	P S	**-58.3**	**-42.8**
13*	56 / F	3m	R > L	+	-	-	-	-	-	n.d.	n.d.	+	-	-	(P S)	**-24.5**	**-47.0**
14	47 / F	1y, 4m	R = L	+	+	+	+	+	-	+	VII, Uln.	+	+	+	P S A	**-16.7**	-8.9
15	65 / M	6m	R > L	+	+	+	+	+	-	+	XI, Uln.	+	-	-	P S	**-29.5**	**-20.4**
16	77 / M	4m	R > L	+	+	-	+	+	-	+	VII	n.d.	-	-	P S A	**-43.7**	**-26.8**
17	68 / M	10y	L > R	+	+	+	+	+	-	+	XI	n.d.	-	+	P S A	**-61.7**	**-41.7**
18	59 / M	8y, 8m	R / -	+	+	+	+	+	-	n.d.	n.d.	+	+	+	P S A	**-16.1**	12.8
19	73 / F	4m	R / -	+	+	+	+	+	n.d.	n.d.	n.d.	+	+	+	P S A	**-22.3**	**-22.4**
20	73 / M	3m	R > L	+	-	+	+	+	-	n.d.	n.d.	+	(-)	-	P S	**-60.7**	**-28.1**
21	66 / M	1y, 9m	L > R	-	+	+	+	-	+	n.d.	n.d.	n.d.	-	+	P S	45.2	12.7
22	48 / M	7m	L > R	-	-	+	+	-	-	+	XI	+	-	-	P S	**-45.2**	**-44.8**
23	54 / M	5y,11m	R > L	+	+	-	+	-	-	n.d.	n.d.	n.d.	+	+	P S A	57.6	85.5
24	85 / M	9y	R / -	+	+	-	+	-	-	n.d.	n.d.	n.d.	-	-	P S	**-32.2**	**-20.4**
25	86 / M	4m	L > R	+	+	+	+	-	-	n.d.	n.d.	+	+	(+)	P A	**-23.2**	**-32.8**
26	81 / M	2y, 6m	L > R	+	+	-	+	n.d.	n.d.	n.d.	n.d.	+	-	-	P S	4.5	**-15.2**
27	38 / F	3y	- / L	-	+	+	-	-	-	+	XI	+	-	-	P S	**-36.3**	**-20.7**

*AChR = acetylcholine receptor; MuSK = muscle-specific kinase; n.d. = not determined; Uln. = ulnar nerve; (-) = unclear mediastinal lesions in CT thorax; (+) = oVEMP was done prior to thymectomy; -/+ = only subjective improvement; P = pyridostigmin; S = steroids; A = azathioprine; (P S) = oVEMP was done prior to treatment; * = isolated ocular myasthenia (patients 1-13); bold = decrement ≥15.2%; underlined = bilateral decrement ≥20.4%.*

Figure 1

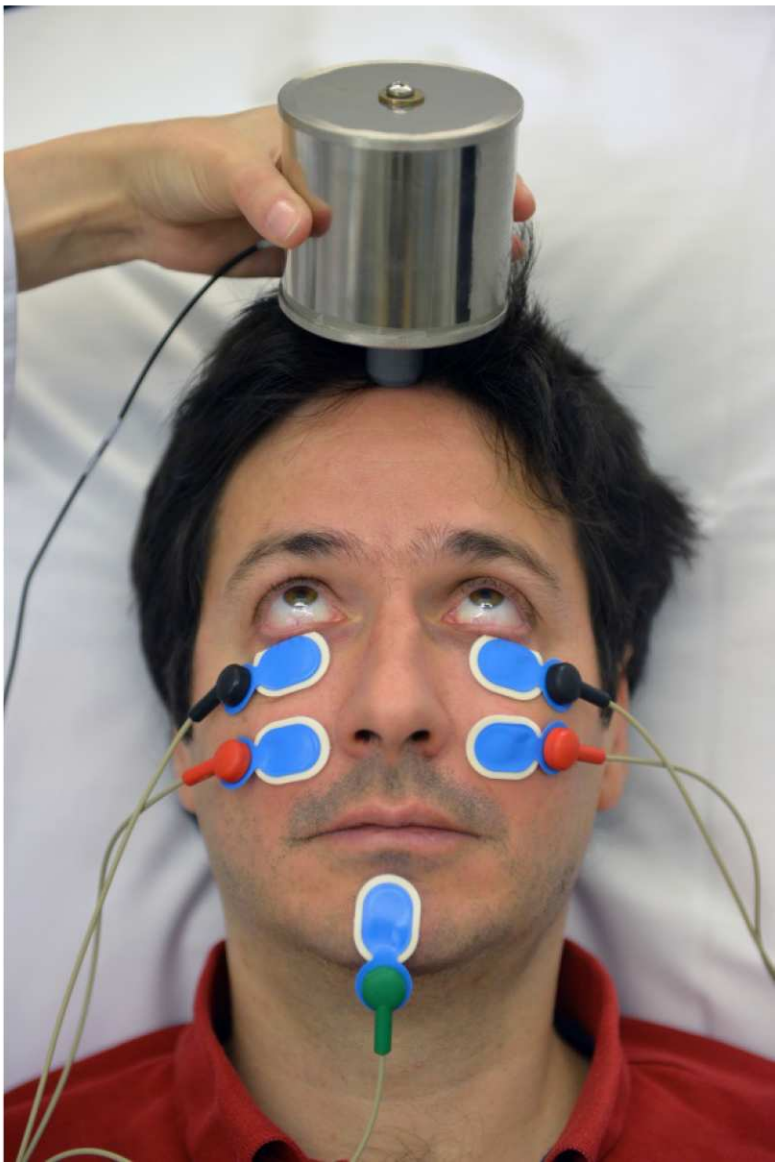


Figure 2

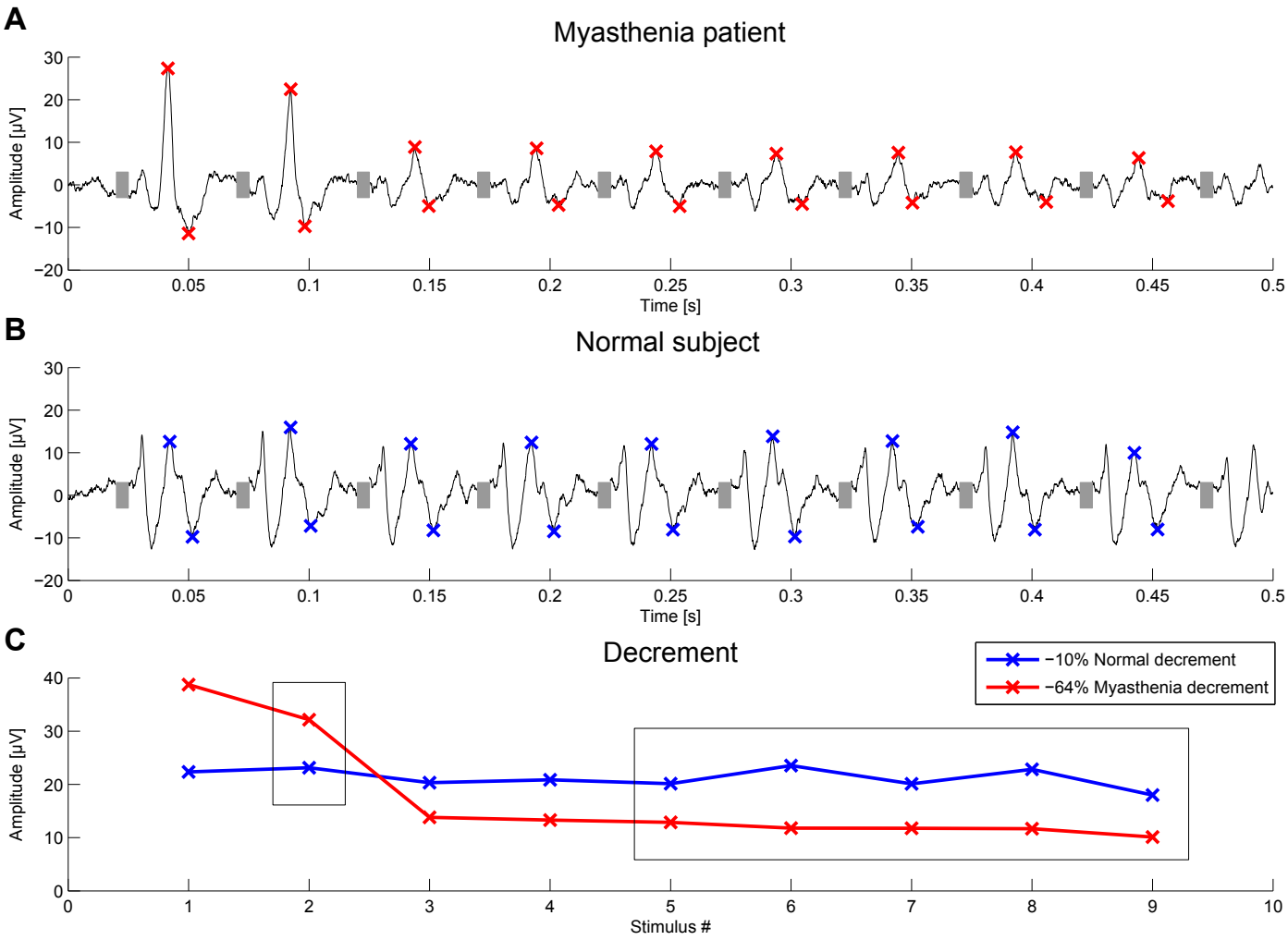

A
Myasthenia patient
Amplitude [μV]
Time [s]
B
Normal subject
Amplitude [μV]
Time [s]
C
Decrement
−10% Normal decrement
−64% Myasthenia decrement
Amplitude [μV]
Stimulus #

Figure 3

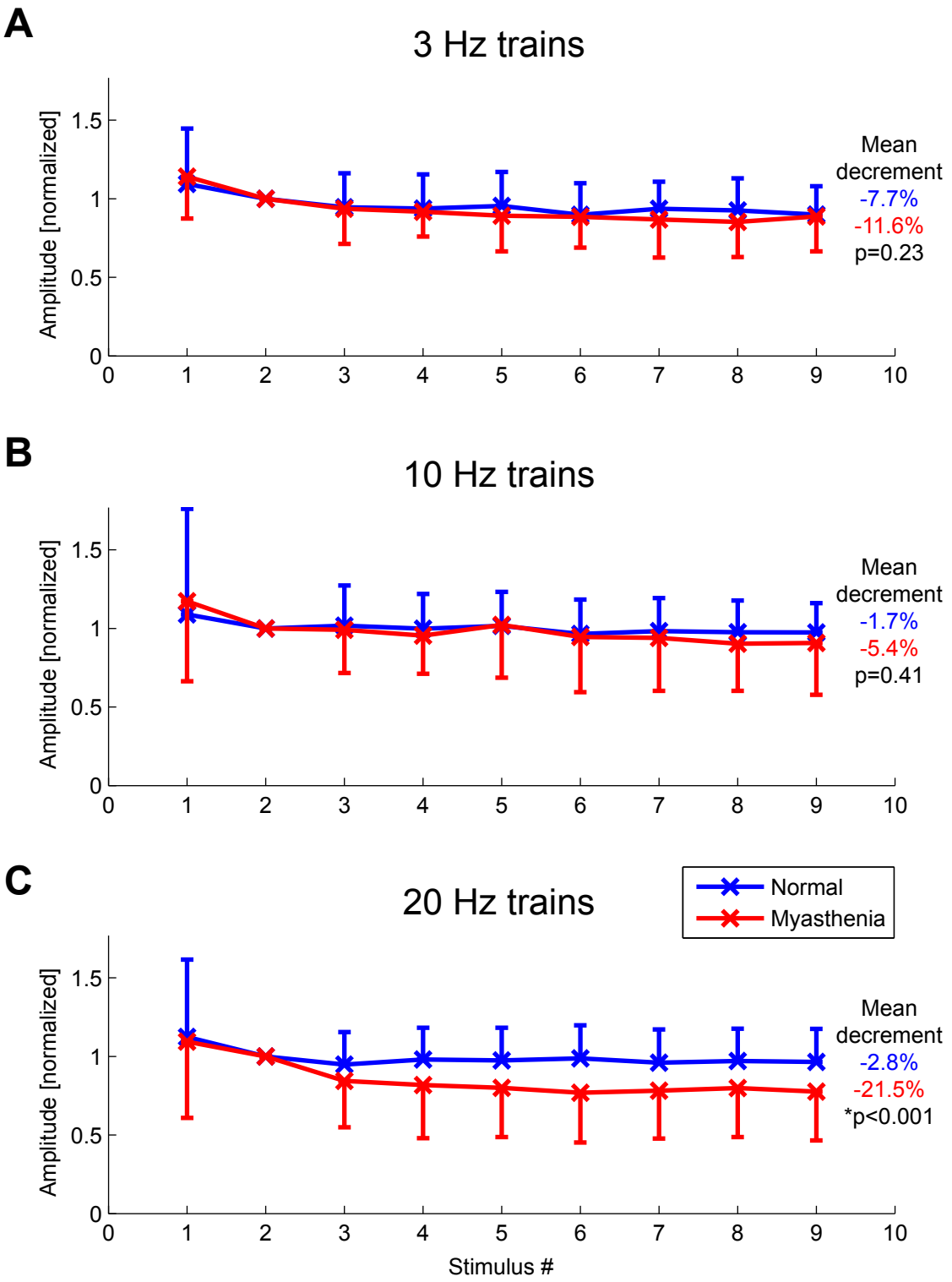
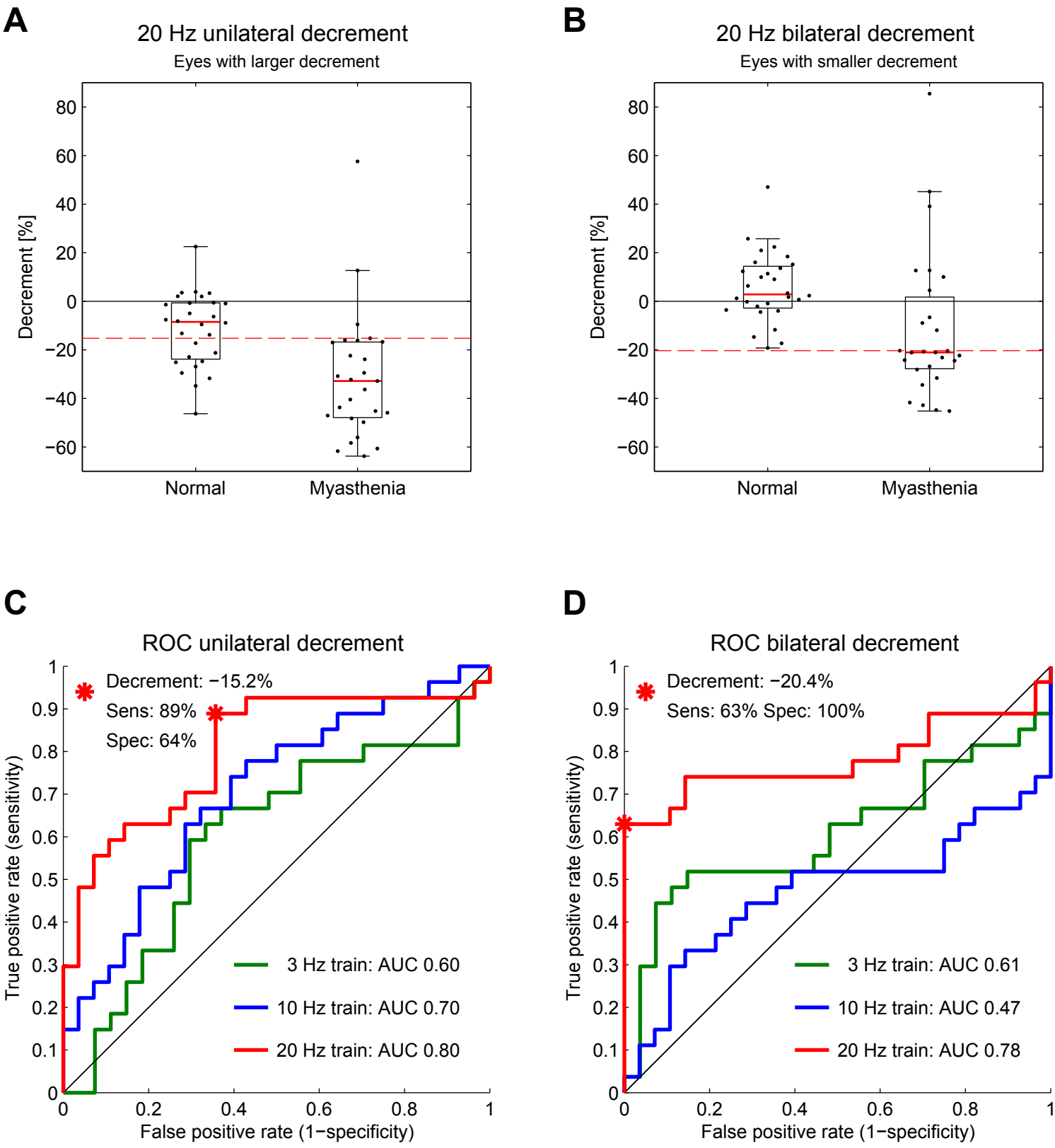

A
3 Hz trains
Amplitude [normalized]
Mean decrement
-7.7%
-11.6%
p=0.23
B
10 Hz trains
Amplitude [normalized]
Mean decrement
-1.7%
-5.4%
p=0.41
C
20 Hz trains
Normal
Myasthenia
Amplitude [normalized]
Mean decrement
-2.8%
-21.5%
*p<0.001
Stimulus #

Figure 4


A
20 Hz unilateral decrement
Eyes with larger decrement
Decrement [%]
80
60
40
20
0
−20
−40
−60
Normal
Myasthenia
B
20 Hz bilateral decrement
Eyes with smaller decrement
Decrement [%]
80
60
40
20
0
−20
−40
−60
Normal
Myasthenia
C
ROC unilateral decrement
Decrement: −15.2%
Sens: 89%
Spec: 64%
3 Hz train: AUC 0.60
10 Hz train: AUC 0.70
20 Hz train: AUC 0.80
True positive rate (sensitivity)
False positive rate (1−specificity)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0.2
0.4
0.6
0.8
D
ROC bilateral decrement
Decrement: −20.4%
Sens: 63% Spec: 100%
3 Hz train: AUC 0.61
10 Hz train: AUC 0.47
20 Hz train: AUC 0.78
True positive rate (sensitivity)
False positive rate (1−specificity)

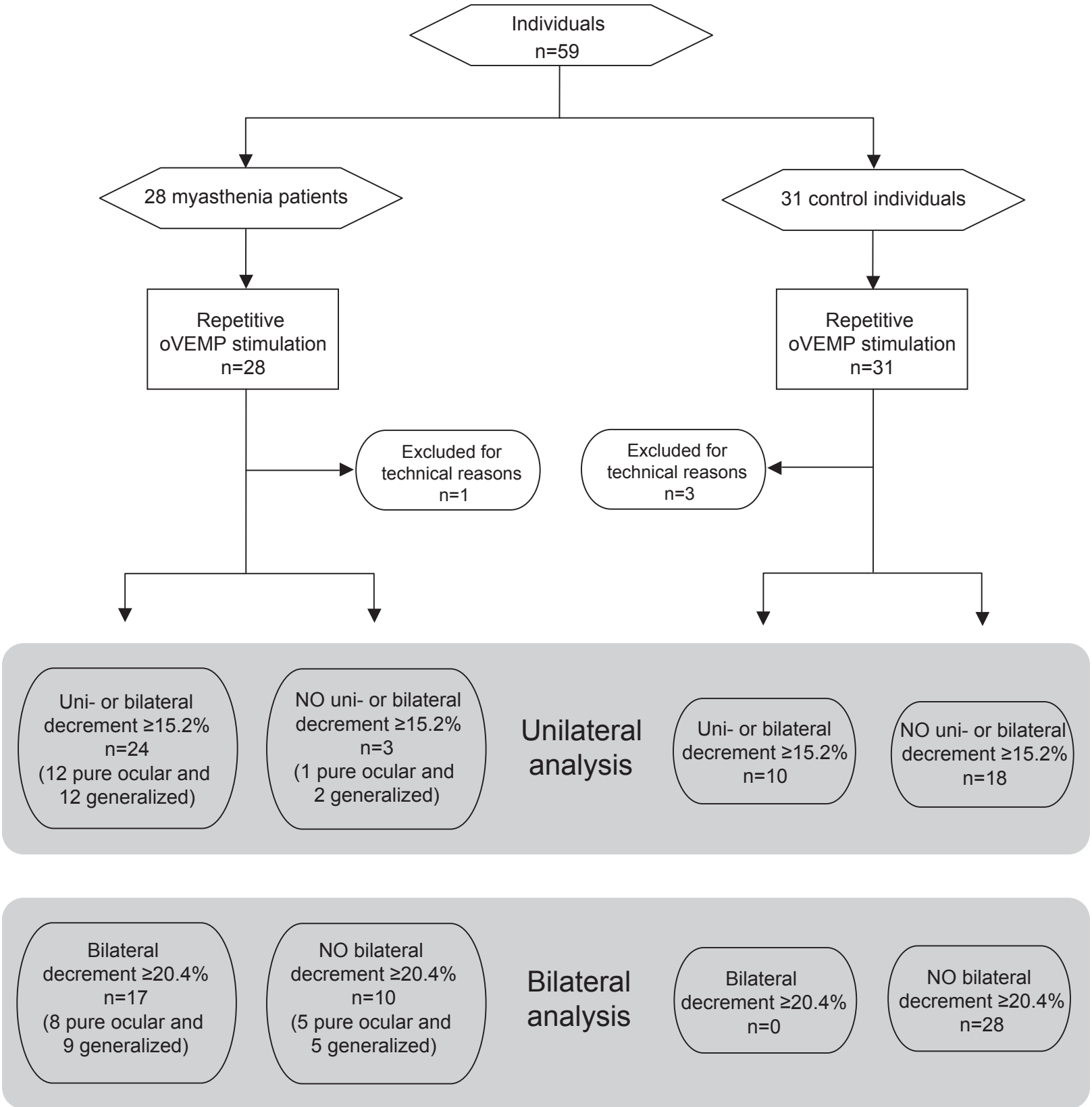


Figure e-1. Study flow diagram.